November 15, 1948.

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Dear Max,

Please excuse my tardiness in answering, but when I returned last Monday from New Haven, I found that I had to recover the heterozygotes again; the stock slants had lost much of their viability, and what was left was mostly segregated. However, this is coming along very well now, and it might be profitable to send them out.

The media now used are a) EMB-lactose (or xylose) :

Peptone (or NZCase)	8
Y. Extract	1
NaCl	5
K_2HPO_4	2
Eosin Y	.4
Methylene Blue	.065
Agar	15
Lactose	10

I usually keep the dyes and phosphate as a dry mix. (EMB mix)

b) Synthetic EMB (EMS)- lactose

Sodium succinate	5
Ammon. sulfate	5
NaCl	1
$MgSO_4$	.1
Agar	15
Lactose	10
EMB mix	2.5

(Asparagine can be used instead if desired)

For "complete" slants: nutrient agar plus .3% yeast extract.
For minimal, (i.e. ~~Bx~~ T(0)) see my paper in Genetics.

When you give me the word that you can start handling cultures, I will send you H-72 which is a prototroph heterozygous for Lac and for Xyl, and for nutritional factors I haven't checked on yet. It was obtained from a cross between B-M-H x T-L-B_1-Lac_1-Mal_1-Gal-Xyl-Arab-V_1^r. H designates the unknown factor which leads to the appearance of heterozygotic prototrophs in crosses and may be a chromosomal aberration. H-72 is not heterozygotic for the other sugars, but is $+$ for them.

You may find handling and preserving these cultures somewhat ticklish at first/ I'm still working on means of alleviating these difficulties. I don't know how lyophilization would work.

You will receive H-72 on a T(0) slant. Immediately upon receipt, the culture should be emulsified in water and streaked out concurrently on EMB and EMS Lac. If the bulk of the cells are still heterozygous, the colonies on EMB will be mosaic + and -; if not they will be intact - or +. On EMS you should get, after 40-50 hours a number of Lac+ colonies. These should be emulsified and streaked in the same way, until you establish a line of transfers which gives + colonies on EMS and mosaics on EMB. The most reliable way of keeping the heterozygotes is by such transfers once or twice weekly. Single colonies serially transferred on EMS, several colonies being chosen from each plate and taken both to EMB and EMS and carrying through the lines which give mosaics on EMB. The line is thus carried on EMS, and the isolates tested on each transfer on EMB. I've carried them this way for 20 or more transfers quite satisfactorily, while mass cultures on slants segregate out very quickly. When the line is established, you will find that only two or three colonies have to be transferred and checked on each transfer to be sure of carrying along a heterozygote.

When heterozygotic colonies are inoculated into complete broth, they rapidly segregate out, and at the end of growth only a very few% will still produce mosaic colonies on EMB. In minimal liquid medium, you may get variable luck; sometimes finding mostly heterozygotes after growth. The most reliable source xr is single colonies from EMS.

I would suggest that before we get together personally, you try your hand at carrying and testing these cultures. Perhaps, it would be well to try to isolate a few single heterozygote cells, to and in minimal medium. That would be a good time for a conference to plan further work. I hope to have a preliminary account of this work written up before many more weeks.

As I don't have any classes, I would be glad to take the <u>time</u> to see you at Ithaca if that were more convenient. But we don't have any funds for that kind of thing, so I could travel in that direction only by invitation. You may be better fixed, in that reppect, to come here.

Your estimate of two weeks to have your lab ready makes me a little envious. I've been here over a year, and have still to move into my new lab, which needs only plumbing and painting now-- another two months probably!

Yours sincerely,

Joshua Lederberg